METHOD 8330A

NITROAROMATICS AND NITRAMINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1.0 SCOPE AND APPLICATION

1.1 Method 8330 is intended for the trace analysis of explosives residues by high performance liquid chromatography using a UV detector. This method is used to determine the concentration of the following compounds in a water, soil, or sediment matrix:

Analyte	Abbreviation	CAS Number
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	2691-41-0
Hexahydro-1,3,5-trinitro-1,3,5-triazine	RDX	121-82-4
1,3,5-Trinitrobenzene	1,3,5-TNB	99-35-4
1,3-Dinitrobenzene	1,3-DNB	99-65-0
Methyl-2,4,6-trinitrophenylnitramine	Tetryl	479-45-8
Nitrobenzene	NB	98-95-3
2,4,6-Trinitrotoluene	2,4,6-TNT	118-96-7
4-Amino-2,6-dinitrotoluene	4-Am-DNT	1946-51-0
2-Amino-4, 6-dinitrotoluene	2-Am-DNT	35572-78-2
2,4-Dinitrotoluene	2,4-DNT	121-14-2
2,6-Dinitrotoluene	2,6-DNT	606-20-2
2-Nitrotoluene	2-NT	88-72-2
3-Nitrotoluene	3-NT	99-08-1
4-Nitrotoluene	4-NT	99-99-0

1.2 Method 8330 provides a salting-out extraction procedure for low concentrations (parts per trillion, or ng/L) of explosives residues in surface or ground water. Direct injection of diluted and filtered water samples can be used for water samples of higher concentration (See Table 1). Solid-phase extraction, using Method 3535, may also be applied to aqueous samples.

1.3 All of these compounds are either used in the manufacture of explosives or are the degradation products of compounds used for that purpose. When making stock solutions for calibration, treat each explosive compound with caution. See NOTE in Sec. 5.3.1 and Sec. 11.

1.4 The estimated quantitation limits (EQLs) of target analytes determined by Method 8330 in water and soil are presented in Table 1.

1.5 This method is restricted to use by or under the supervision of analysts experienced in the use of HPLC, skilled in the interpretation of chromatograms, and experienced in handling explosive materials. (See Sec. 11.0 on SAFETY.) Each analyst must demonstrate the ability to generate acceptable results with this method.

2.0 SUMMARY OF METHOD

2.1 Method 8330 provides high performance liquid chromatographic (HPLC) conditions for the detection of ppb levels of certain explosive residues in water, soil, and sediment. Prior to use of this method, appropriate sample preparation techniques must be used.

2.2 Low-level salting-out method with no evaporation - Aqueous samples of low concentration are extracted by a salting-out extraction procedure with acetonitrile and sodium chloride. The small volume of acetonitrile that remains undissolved above the salt water is drawn off and transferred to a smaller volumetric flask. It is back-extracted by vigorous stirring with a specific volume of salt water. After equilibration, the phases are allowed to separate and the small volume of acetonitrile residing in the narrow neck of the volumetric flask is removed using a Pasteur pipet. The concentrated extract is diluted 1:1 with reagent grade water. An aliquot is separated on a C-18 reversed-phase column, determined at 254 nm, and confirmed on a CN reversed-phase column.

2.3 Solid-phase extraction method - Aqueous samples may also be prepared using solid-phase extraction, as described in Method 3535.

2.4 High-level direct injection method - Aqueous samples of higher concentration can be diluted 1/1 (v/v) with methanol or acetonitrile, filtered, separated on a C-18 reversed-phase column, determined at 254 nm, and confirmed on a CN reversed-phase column. If HMX is an important target analyte, methanol is preferred.

2.5 Soil and sediment samples are extracted using acetonitrile in an ultrasonic bath, filtered and analyzed as described in Sec. 2.3.

3.0 INTERFERENCES

3.1 Solvents, reagents, glassware and other sample processing hardware may yield discrete artifacts and/or elevated baselines, causing misinterpretation of the chromatograms. All of these materials must be demonstrated to be free from interferences.

3.2 2,4-DNT and 2,6-DNT elute at similar retention times (retention time difference of 0.2 minutes). A large concentration of one isomer may mask the response of the other isomer. If it is not apparent that both isomers are present (or are not detected), an isomeric mixture should be reported.

3.3 Tetryl decomposes rapidly in methanol/water solutions, as well as with heat. All aqueous samples expected to contain tetryl should be diluted with acetonitrile prior to filtration and acidified to pH <3. All samples expected to contain tetryl should not be exposed to temperatures above room temperature.

3.4 Degradation products of tetryl appear as a shoulder on the 2,4,6-TNT peak. Peak heights rather than peak areas should be used when tetryl is present in concentrations that are significant relative to the concentration of 2,4,6-TNT.

4.0 APPARATUS AND MATERIALS

4.1 HPLC system

4.1.1 HPLC - equipped with a pump capable of achieving 4000 psi, a 100-µL loop injector and a 254-nm UV detector (Perkin-Elmer Series 3, or equivalent). For the low concentration option, the detector must be capable of maintaining a stable baseline at 0.001 absorbance units full scale.

4.1.2 Recommended columns

4.1.2.1 Primary column - C-18 Reversed-phase HPLC column, 25-cm x 4.6-mm (5 µm) (Supelco LC-18, or equivalent).

4.1.2.2 Secondary column - CN Reversed-phase HPLC column, 25-cm x 4.6-mm (5 µm) (Supelco LC-CN, or equivalent).

4.1.3 Strip chart recorder

4.1.4 Digital integrator (optional)

4.1.5 Autosampler (optional)

4.2 Other equipment

4.2.1 Temperature-controlled ultrasonic bath

4.2.2 Vortex mixer

4.2.3 Balance - capable of weighing ± 0.0001 g

4.2.4 Magnetic stirrer with PTFE stirring bars

4.2.5 Water bath - Heated, with concentric ring cover, capable of temperature control (± 5°C). The bath should be used in a hood.

4.2.6 Oven - Forced air, without heating.

4.3 Materials

4.3.1 High-pressure injection syringe - 500-µL (Hamilton liquid syringe, or equivalent).

4.3.2 Disposable cartridge filters - 0.45-µm PTFE filter.

4.3.3 Pipets - Class A, glass, appropriate sizes.

4.3.4 Pasteur pipets

4.3.5 Scintillation vials - 20-mL, glass.

4.3.6 Vials - 15-mL, glass, PTFE-lined cap.

4.3.7 Vials - 40-mL, glass, PTFE-lined cap.

4.3.8 Disposable syringes - Plastipak, 3-mL and 10-mL or equivalent.

4.3.9 Volumetric flasks - 10-mL, 25-mL, 100-mL, and 1-L, with ground-glass stoppers, Class A.

NOTE: The 100-mL and 1-L volumetric flasks used for magnetic stirrer extraction must be round.

4.3.10 Vacuum desiccator - Glass.

4.3.11 Mortar and pestle - Steel.

4.3.12 Sieve - 30-mesh.

4.3.13 Graduated cylinders - 10-mL, 25-mL, and 1-L.

5.0 REAGENTS

5.1 Reagent grade inorganic chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lowering the accuracy of the determination.

5.1.1 Acetonitrile, CH_3CN - HPLC grade.

5.1.2 Methanol, CH_3OH - HPLC grade.

5.1.3 Calcium chloride, $CaCl_2$ - Reagent grade. Prepare an aqueous solution containing 5 g/L of calcium chloride.

5.1.4 Sodium chloride, NaCl, shipped in glass bottles - reagent grade.

5.2 Organic-free reagent water - All references to water in this method refer to organic-free reagent water, as defined in Chapter One.

5.3 Stock standard solutions

Dry each solid analyte standard to constant weight in a vacuum desiccator in the dark. Place about 0.100 g (weighed to 0.0001 g) of a single analyte into a 100-mL volumetric flask and dilute to volume with acetonitrile. Invert flask several times until dissolved. Store in refrigerator at 4°C in the dark. Calculate the concentration of the stock solution from the actual weight used (nominal concentration = 1,000 mg/L). Stock solutions may be used for up to one year.

NOTE: The HMX, RDX, Tetryl, and 2,4,6-TNT are explosives and the neat material should be handled carefully. See SAFETY in Sec. 11 for guidance. HMX, RDX, and Tetryl reference materials are shipped under water. Drying at ambient temperature requires several days. DO NOT DRY AT ELEVATED TEMPERATURES!

5.4 Intermediate standards solutions

5.4.1 If both 2,4-DNT and 2,6-DNT are to be determined, prepare two separate intermediate stock solutions containing (1) HMX, RDX, 1,3,5-TNB, 1,3-DNB, NB, 2,4,6-TNT, and 2,4-DNT and (2) Tetryl, 2,6-DNT, 2-NT, 3-NT, and 4-NT. Intermediate stock standard solutions should be prepared at 1,000 µg/L, in acetonitrile when analyzing soil samples, and in methanol when analyzing aqueous samples.

5.4.2 Dilute the two concentrated intermediate stock solutions, with the appropriate solvent, to prepare intermediate standard solutions that cover the range of 2.5 - 1,000 µg/L. These solutions should be refrigerated on preparation, and may be used for 30 days.

5.4.3 For the low-level method, the analyst must conduct a detection limit study and devise dilution series appropriate to the desired range. Standards for the low level method must be prepared immediately prior to use.

5.5 Working standards - Calibration standards at a minimum of five concentration levels should be prepared by the dilution of the intermediate standards solutions by 50% (v/v) with 5 g/L calcium chloride solution (Sec. 5.1.3). These solutions must be refrigerated and stored in the dark, and prepared fresh on the day of calibration.

5.6 Surrogate spiking solution - The analyst should monitor the performance of the extraction and analytical system as well as the effectiveness of the method in dealing with each sample matrix by spiking each sample, standard and reagent water blank with one or two surrogates (e.g., analytes not expected to be present in the sample).

5.7 Matrix spiking solutions - Prepare matrix spiking solutions in methanol such that the concentration in the sample is five times the Estimated Quantitation Limit (Table 1). All target analytes should be included.

5.8 HPLC mobile phase - To prepare 1 L of mobile phase, add 500 mL of methanol to 500 mL of organic-free reagent water.

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 Follow conventional sampling and sample handling procedures as specified for semivolatile organics in Chapter Four.

6.2 Samples and sample extracts must be stored in the dark at 4°C. Holding times are the same as for semivolatile organics.

7.0 PROCEDURE

7.1 Sample preparation

This method addresses both aqueous and solid samples. There are three extraction procedures that may be applied to aqueous samples, depending on the expected level of explosive residue in the sample and the available equipment: a low-level salting-out extraction, a high-level extraction, and solid-phase extraction. It is highly recommended that aqueous process waste samples be screened with the high-level method to determine if the low-level method (1-50 µg/L) is required. Most groundwater samples will fall into the low-level method.

7.1.1 Aqueous low-level method (salting-out extraction)

7.1.1.1 Add 251.3 g of sodium chloride to a 1-L volumetric flask (round). Measure 770 mL of a water sample (using a 1-L graduated cylinder) and transfer it to the volumetric flask containing the salt. Add a stir bar and mix the contents at maximum speed on a magnetic stirrer until the salt is completely dissolved.

7.1.1.2 Add 164 mL of acetonitrile (measured with a 250-mL graduated cylinder) while the solution is being stirred and stir for an additional 15 minutes. Turn off the stirrer and allow the phases to separate for 10 minutes.

7.1.1.3 Remove the acetonitrile (upper) layer (about 8 mL) with a Pasteur pipet and transfer it to a 100-mL volumetric flask (with a round bottom). Add 10 mL of fresh acetonitrile to the water sample in the 1-L flask. Again stir the contents of the flask for 15 minutes followed by 10 minutes for phase separation. Combine the second acetonitrile portion with the initial extract. The inclusion of a few drops of salt water at this point is unimportant.

7.1.1.4 Add 84 mL of salt water (325 g NaCl per 1000 mL of reagent water) to the acetonitrile extract in the 100-mL volumetric flask. Add a stir bar and stir the contents on a magnetic stirrer for 15 minutes, followed by 10 minutes for phase separation. Carefully transfer the acetonitrile phase to a 10-mL graduated cylinder using a Pasteur pipet. At this stage, the amount of water transferred with the acetonitrile must be minimized. The water contains a high concentration of NaCl that produces a large peak at the beginning of the chromatogram, where it could interfere with the HMX determination.

7.1.1.5 Add an additional 1.0 mL of acetonitrile to the 100-mL volumetric flask. Again stir the contents of the flask for 15 minutes, followed by 10 minutes for phase separation. Combine the second acetonitrile portion with the initial extract in the 10-mL graduated cylinder (transfer to a 25-mL graduated cylinder if the volume exceeds 5 mL). Record the total volume of acetonitrile extract to the nearest 0.1 mL. (Use this as the volume of total extract [V_t] in the calculation of concentration after converting to µL). The resulting extract, about 5 - 6 mL, is then diluted 1:1 with organic-free reagent water (with pH <3 if tetryl is a suspected analyte) prior to analysis.

7.1.1.6 If the diluted extract is turbid, filter it through a 0.45-µm PTFE filter using a disposable syringe. Discard the first 0.5 mL of filtrate, and retain the remainder in a PTFE-capped vial for RP-HPLC analysis in Sec. 7.4.

7.1.2 Aqueous high-level method

7.1.2.1 Sample filtration

Place a 5-mL aliquot of each water sample in a scintillation vial, add 5 mL of acetonitrile, shake thoroughly, and filter through a 0.45-µm PTFE filter using a disposable syringe.

7.1.2.2 Discard the first 3 mL of filtrate, and retain the remainder in a PTFE-capped vial for RP-HPLC analysis in Sec. 7.4. HMX quantitation can be improved with the use of methanol rather than acetonitrile for dilution before filtration.

7.1.3 Solid-phase extraction

Aqueous samples containing nitroaromatics and nitramines may also be extracted using solid-phase extraction (SPE) in both disk and cartridge formats. Consult Method 3535 for the procedures to be employed and the apparatus and materials that are required.

7.1.4 Soil and sediment samples

7.1.4.1 Sample homogenization

Dry soil samples in air at room temperature (or less) to a constant weight, being careful not to expose the samples to direct sunlight. Grind and homogenize the dried sample thoroughly in an acetonitrile-rinsed mortar to pass a 30-mesh sieve.

NOTE: Soil samples should be screened by Method 8515 prior to grinding in a mortar and pestle (See Safety Sec. 11.2).

7.1.4.2 Sample extraction

7.1.4.2.1 Place a 2.0-g subsample of each soil sample in a 15-mL glass vial. Add 10.0 mL of acetonitrile, cap with PTFE-lined cap, vortex swirl for one minute, and place in a cooled ultrasonic bath for 18 hours.

7.1.4.2.2 After sonication, allow sample to settle for 30 minutes. Remove 5.0 mL of supernatant, and combine with 5.0 mL of calcium chloride solution (Sec. 5.1.3) in a 20-mL vial. Shake, and let stand for 15 minutes.

7.1.4.2.3 Place supernatant in a disposable syringe and filter through a 0.45-µm PTFE filter. Discard first 3 mL and retain remainder in a PTFE-capped vial for RP-HPLC analysis in Sec. 7.4.

7.2 Chromatographic conditions (recommended)

Primary Column: C-18 reversed-phase HPLC column, 25-cm x 4.6-mm, 5 µm (Supelco LC-18 or equivalent).

Secondary Column: CN reversed-phase HPLC column, 25-cm x 4.6-mm, 5 µm (Supelco LC-CN or equivalent).

Mobile Phase: 50/50 (v/v) methanol/organic-free reagent water.

Flow Rate: 1.5 mL/min

Injection volume: 100-µL

UV Detector: 254 nm

7.3 Calibration of HPLC

7.3.1 All electronic equipment is allowed to warm up for 30 minutes. During this period, at least 15 void volumes of mobile phase are passed through the column (approximately 20 min at 1.5 mL/min) and continued until the baseline is level at the UV detector's greatest sensitivity.

7.3.2 Initial calibration - Injections of each calibration standard over the concentration range of interest are made sequentially into the HPLC in random order. Peak heights or peak areas are obtained for each analyte. Employ one of the calibration options described in Method 8000.

7.3.3 Calibration verification - Analyze one mid-point calibration standard, at a minimum, at the beginning of the day, and after every 20 sample extracts (*recommended* after every 10, in order to minimize the number of samples that may be affected by a failing standard), and after the last sample of the day. Calculate the calibration factor for each analyte from the peak height or peak area and compare it with the mean calibration factor obtained for the initial calibration, as described in Method 8000. The calibration factor for the calibration verification must agree within ±15% of the mean calibration factor of the initial calibration. If this criterion is not met, a new initial calibration must be performed, or another of the calibration options described in Method 8000 must be employed.

7.4 HPLC analysis

7.4.1 Analyze the samples using the chromatographic conditions given in Sec. 7.2. All positive measurements observed on the C-18 column must be confirmed by injection onto the CN column.

7.4.2 Method 8000 provides instructions on the analysis sequence, appropriate dilutions, establishing daily retention time windows, and identification criteria. Include a mid-level standard after each group of 20 samples in the analysis sequence. If column temperature control is not employed, special care must be taken to ensure that temperature shifts do not cause peak misidentification.

7.4.3 Table 2 summarizes the estimated retention times on both C-18 and CN columns for a number of analytes analyzable using this method. An example of the separation achieved by Column 1 is shown in Figure 1.

7.4.4 Record the resulting peak sizes in peak heights or area units. The use of peak heights is recommended to improve reproducibility of low level samples.

7.4.5 The calculation of sample concentrations is described in Method 8000.

8.0 QUALITY CONTROL

8.1 Refer to Chapter One and Method 8000 for specific quality control (QC) procedures. Quality control procedures to ensure the proper operation of the various sample preparation and/or sample introduction techniques can be found in Method 3500. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated.

8.2 Quality control procedures that are necessary to validate the HPLC system operation are found in Method 8000, Sec. 8.0.

8.3 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes, by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the following operations whenever new staff are trained or significant changes in instrumentation are made. See Method 8000, Sec. 8.0 for information on how to accomplish this demonstration.

8.4 Sample quality control for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, and quantitation limit). At a minimum, this includes the analysis of QC samples including a method blank, matrix spike, a duplicate, and a laboratory control sample (LCS) in each analytical batch and the addition of surrogates to each field sample and QC sample.

8.4.1 Before processing any samples, the analyst should demonstrate, through the analysis of a method blank, that interferences from the analytical system, glassware, and reagents are under control. Each time a set of samples is analyzed or there is a change in reagents, a method blank should be analyzed as a safeguard against chronic laboratory contamination. The blanks should be carried through all stages of sample preparation and measurement.

8.4.2 Documenting the effect of the matrix should include the analysis of at least one matrix spike and one duplicate unspiked sample or one matrix spike/matrix spike duplicate pair. The decision on whether to prepare and analyze duplicate samples or a matrix spike/matrix spike duplicate must be based on a knowledge of the samples in the sample batch. If samples are expected to contain target analytes, then laboratories may use one matrix spike and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, laboratories should use a matrix spike and matrix spike duplicate pair.

8.4.3 A Laboratory Control Sample (LCS) should be included with each analytical batch. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume. The LCS is spiked with the same analytes at the same concentrations as the matrix spike. When the results of the matrix spike analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix.

8.4.4 See Method 8000, Sec. 8.0, for the details on carrying out sample quality control procedures for preparation and analysis.

8.5 Surrogate recoveries

The laboratory must evaluate surrogate recovery data from individual samples versus the surrogate control limits developed by the laboratory. See Method 8000, Sec. 8.0, for information on evaluating surrogate data and developing and updating surrogate limits.

8.6 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

9.0 METHOD PERFORMANCE

9.1 Table 3 provides the single-laboratory precision based on data from the analysis of blind duplicates of four spiked soil samples and four field-contaminated samples analyzed by seven laboratories.

9.2 Table 4 provides the multi-laboratory error based on data from the analysis of blind duplicates of four spiked soil samples and four field-contaminated samples analyzed by seven laboratories.

9.3 Table 5 provides the multi-laboratory variance of the high-level method for water based on data from nine laboratories.

9.4 Table 6 provides multi-laboratory recovery data from the analysis of spiked soil samples by seven laboratories.

9.5 Table 7 provides a comparison of method accuracy for soil and aqueous samples (high-level method).

9.6 Table 8 provides precision and accuracy data for the salting-out extraction method.

9.7 Table 9 provides data from a comparison of direct injection of groundwater samples with both the salting-out extraction and the solid-phase extraction techniques.

9.8 Table 10 provides data comparing the precision of duplicate samples analyzed by direct injection of groundwater samples with both the salting-out extraction and the solid-phase extraction techniques.

9.9 Table 11 provides a comparison of recovery data for spiked samples analyzed by direct injection of groundwater samples with both the salting-out extraction and the solid-phase extraction techniques.

10.0 REFERENCES

1. Bauer, C.F., T.F. Jenkins, S.M. Koza, P.W. Schumacher, P.H. Miyares and M.E. Walsh (1989). Development of an analytical method for the determination of explosive residues in soil. Part 3. Collaborative test results and final performance evaluation. USACE Cold Regions Research and Engineering Laboratory, CRREL Report 89-9.

2. Grant, C.L., A.D. Hewitt and T.F. Jenkins (1989). Comparison of low concentration measurement capability estimates in trace analysis: Method Detection Limits and Certified Reporting Limits. USACE Cold Regions Research and Engineering Laboratory, Special Report 89-20.

3. Jenkins, T.F., C.F. Bauer, D.C. Leggett and C.L. Grant (1984). Reverse-phased HPLC method for analysis of TNT, RDX, HMX and 2,4-DNT in munitions wastewater. USACE Cold Regions Research and Engineering Laboratory, CRREL Report 84-29.

4. Jenkins, T.F. and M.E. Walsh (1987). Development of an analytical method for explosive residues in soil. USACE Cold Regions Research and Engineering Laboratory, CRREL Report 87-7.

5. Jenkins, T.F., P.H. Miyares and ME. Walsh (1988a). An improved RP-HPLC method for determining nitroaromatics and nitramines in water. USACE Cold Regions Research and Engineering Laboratory, Special Report 88-23.

6. Jenkins, T.F. and P.H. Miyares (1992). Comparison of Cartridge and Membrane Solid-Phase Extraction with Salting-out Solvent Extraction for Preconcentration of Nitroaromatic and Nitramine Explosives from Water. USACE Cold Regions Research and Engineering Laboratory, Draft CRREL Special Report.

7. Jenkins, T.F., P.W. Schumacher, M.E. Walsh and C.F. Bauer (1988b). Development of an analytical method for the determination of explosive residues in soil. Part II: Further development and ruggedness testing. USACE Cold Regions Research and Engineering Laboratory, CRREL Report 88-8.

8. Leggett, D.C., T.F. Jenkins and P.H. Miyares (1990). Salting-out solvent extraction for preconcentration of neutral polar organic solutes from water. Analytical Chemistry, 62: 1355-1356.

9. Miyares, P.H. and T.F. Jenkins (1990). Salting-out solvent extraction for determining low levels of nitroaromatics and nitramines in water. USACE Cold Regions Research and Engineering Laboratory, Special Report 90-30.

10. Jenkins, T. F., Thorne, P. G., Myers, K. F., McCormick, E. F., Parker, D. E., and B. L. Escalon (1995). Evaluation of Clean Solid Phases for Extraction of Nitroaromatics and Nitramines from Water. USACE Cold Regions Research and Engineering Laboratory, Special Report 95-22.

11.0 SAFETY

11.1 Standard precautionary measures used for handling other organic compounds should be sufficient for the safe handling of the analytes targeted by Method 8330. The only extra caution that should be taken is when handling the analytical standard neat material for the explosives themselves and in rare cases where soil or waste samples are highly contaminated with the explosives. Follow the note for drying the neat materials at ambient temperatures.

11.2 It is advisable to screen soil or waste samples using Method 8515 to determine whether high concentrations of explosives are present. Soil samples containing as much as 2% of 2,4,6-TNT have been safely ground. Samples containing higher concentrations should not be ground in the mortar and pestle. Method 8515 is for 2,4,6-TNT, however, the other nitroaromatics will also cause

a color to be developed and provide a rough estimation of their concentrations. 2,4,6-TNT is the analyte most often detected in high concentrations in soil samples. Visual observation of a soil sample is also important when the sample is taken from a site expected to contain explosives. Lumps of material that have a chemical appearance should be suspect and not ground. Explosives are generally a very finely ground grayish-white material.

TABLE 1

ESTIMATED QUANTITATION LIMITS

	Water (µg/L)		
Analytes	Low-Level	High-Level	Soil (mg/kg)
HMX	-	13.0	2.2
RDX	0.84	14.0	1.0
1,3,5-TNB	0.26	7.3	0.25
1,3-DNB	0.11	4.0	0.25
Tetryl	-	4.0	0.65
NB	-	6.4	0.26
2,4,6-TNT	0.11	6.9	0.25
4-Am-DNT	0.060	-	-
2-Am-DNT	0.035	-	-
2,6-DNT	0.31	9.4	0.26
2,4-DNT	0.020	5.7	0.25
2-NT	-	12.0	0.25
4-NT	-	8.5	0.25
3-NT	-	7.9	0.25

TABLE 2

RETENTION TIMES AND CAPACITY FACTORS ON LC-18 AND LC-CN COLUMNS

	Retention time (min)		Capacity factor (k)*	
Analyte	LC-18	LC-CN	LC-18	LC-CN
HMX	2.44	8.35	0.49	2.52
RDX	3.73	6.15	1.27	1.59
1,3,5-TNB	5.11	4.05	2.12	0.71
1,3-DNB	6.16	4.18	2.76	0.76
Tetryl	6.93	7.36	3.23	2.11
NB	7.23	3.81	3.41	0.61
2,4,6-TNT	8.42	5.00	4.13	1.11
4-Am-DNT	8.88	5.10	4.41	1.15
2-Am-DNT	9.12	5.65	4.56	1.38
2,6-DNT	9.82	4.61	4.99	0.95
2,4-DNT	10.05	4.87	5.13	1.05
2-NT	12.26	4.37	6.48	0.84
4-NT	13.26	4.41	7.09	0.86
3-NT	14.23	4.45	7.68	0.88

*Capacity factors are based on an unretained peak for nitrate at 1.71 min on LC-18 and at 2.00 min on LC-CN.

TABLE 3

SINGLE LABORATORY PRECISION OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
Analyte	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	1.7	3.7	14	1.8	12.8
				153	21.6	14.1
RDX	60	1.4	2.3	104	12	11.5
				877	29.6	3.4
1,3,5-TNB	8.6	0.4	4.6	2.8	0.2	7.1
	46	1.9	4.1	72	6.0	8.3
2,4,6-TNT	40	1.4	3.5	7.0	0.61	9.0
				669	55	8.2
1,3-DNB	3.5	0.14	4.0	1.1	0.11	9.8
2,4-DNT	5.0	0.17	3.4	1.0	0.44	42.3
Tetryl	17	3.1	17.9	2.3	0.41	18.0

Source: Reference 1.

TABLE 4

MULTILABORATORY ERROR OF METHOD FOR SOIL SAMPLES

	Spiked Soils			Field-Contaminated Soils		
Analyte	Mean Conc. (mg/kg)	SD	%RSD	Mean Conc. (mg/kg)	SD	%RSD
HMX	46	2.6	5.7	14	3.7	26.0
				153	37.3	24.0
RDX	60	2.6	4.4	104	17.4	17.0
				877	67.3	7.7
1,3,5-TNB	8.6	0.61	7.1	2.8	0.23	8.2
	46	2.97	6.5	72	8.8	12.2
2,4,6-TNT	40	1.88	4.7	7.0	1.27	18.0
				669	63.4	9.5
1,3-DNB	3.5	0.24	6.9	1.1	0.16	14.5
2,4-DNT	5.0	0.22	4.4	1.0	0.74	74.0
Tetryl	17	5.22	30.7	2.3	0.49	21.3

Source: Reference 1.

TABLE 5

MULTILABORATORY VARIANCE OF METHOD FOR WATER SAMPLES[a]

Analyte	Mean Conc. (µg/L)	SD	%RSD
HMX	203	14.8	7.3
RDX	274	20.8	7.6
2,4-DNT	107	7.7	7.2
2,4,6-TNT	107	11.1	10.4

[a] Nine Laboratories

TABLE 6

MULTILABORATORY RECOVERY DATA FOR SPIKED SOIL SAMPLES

	Concentration (µg/g)						
Laboratory	HMX	RDX	1,3,5-TNB	1,3-DNB	Tetryl	2,4,6-TNT	2,4-DNT
1	44.97	48.78	48.99	49.94	32.48	49.73	51.05
3	50.25	48.50	45.85	45.96	47.91	46.25	48.37
4	42.40	44.00	43.40	49.50	31.60	53.50	50.90
5	46.50	48.40	46.90	48.80	32.10	55.80	49.60
6	56.20	55.00	41.60	46.30	13.20	56.80	45.70
7	41.50	41.50	38.00	44.50	2.60	36.00	43.50
8	52.70	52.20	48.00	48.30	44.80	51.30	49.10
True Conc	50.35	50.20	50.15	50.05	50.35	50.65	50.05
Mean Conc	47.79	48.34	44.68	47.67	29.24	49.91	48.32
Std. Dev.	5.46	4.57	3.91	2.09	16.24	7.11	2.78
% RSD	11.42	9.45	8.75	4.39	55.53	14.26	5.76
% Diff.*	5.08	3.71	10.91	4.76	41.93	1.46	3.46
Mean % Recovery	95	96	89	95	58	98	96

* Between true value and mean determined value.
Source: Reference 1.

TABLE 7

COMPARISON OF METHOD ACCURACY FOR SOIL AND AQUEOUS SAMPLES
(HIGH CONCENTRATION METHOD)

	Recovery (%)	
Analyte	Soil Method*	Aqueous Method**
2,4-DNT	96.0	98.6
2,4,6-TNT	96.8	94.4
RDX	96.8	99.6
HMX	95.4	95.5

* Data from Reference 1.
** Data from Reference 3.

TABLE 8

PRECISION AND ACCURACY DATA FOR THE SALTING-OUT EXTRACTION METHOD

Analyte	# Samples	%RSD	Mean Recovery (%)	Highest Concentration Tested
HMX	20	10.5	106	1.14
RDX	20	8.7	106	1.04
1,3,5-TNB	20	7.6	119	0.82
1,3-DNB	20	6.6	102	1.04
Tetryl	20	16.4	93	0.93
2,4,6-TNT	20	7.6	105	0.98
2-Am-DNT	20	9.1	102	1.04
2,4-DNT	20	5.8	101	1.01
1,2-NT	20	9.1	102	1.07
1,4-NT	20	18.1	96	1.06
1,3-NT	20	12.4	97	1.23

All tests were performed in reagent water.
Source: Reference 6.

TABLE 9

COMPARISON OF DIRECT ANALYSIS OF GROUNDWATER SAMPLES CONTAINING NITROAROMATICS WITH SALTING-OUT AND SOLID-PHASE EXTRACTION TECHNIQUES

		Analyte Concentration (µg/L)								
Sample	Technique	HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
1	Direct									
	Salting-out	1.04	2.45				0.47		0.36	0.32
	SPE-Cart.	1.00	1.33				0.44		0.29	0.30
	SPE-Disk	0.93	1.35				0.57		0.28	0.56
2	Direct	94	79							
	Salting-out	54.2	63.8			0.3	0.33		3.08	1.36
	SPE-Cart.	64.0	83.1			0.3	0.34		3.34	2.27
	SPE-Disk	57.1	71.8			0.3	0.29		2.89	2.05
3	Direct	93	91							
	Salting-out	85.7	75.3			0.2	0.19		2.43	1.31
	SPE-Cart.	93.1	88.8			0.2	0.17		2.49	1.65
	SPE-Disk	78.9	74.7			0.2	0.13		1.99	1.89
4	Direct	45	14							
	Salting-out	45.7	16.4		0.17	0.3	0.13		2.18	1.21
	SPE-Cart.	48.0	21.6			0.2	0.19		2.31	1.42
	SPE-Disk	40.8	18.9			0.2	0.13		2.07	1.64
5	Direct									
	Salting-out	0.76	5.77						0.13	0.05
	SPE-Cart.	1.16	6.48						0.16	0.05
	SPE-Disk	1.19	6.11						0.16	0.14
6	Direct									
	Salting-out	10.5	6.17				0.10		0.71	0.33
	SPE-Cart.	11.5	7.03				0.10		0.79	0.40
	SPE-Disk	10.3	6.34				0.07		0.82	0.70

TABLE 9
(continued)

		Analyte Concentration (µg/L)								
Sample	Technique	HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
7	Direct	134	365							
	Salting-out	75.4	202				0.98		8.12	1.80
	SPE-Cart.	115	308				1.51		11.3	3.44
	SPE-Disk	109	291				1.41		9.81	3.30
8	Direct									
	Salting-out	0.61	10.9							
	SPE-Cart.	0.64	11.9							
	SPE-Disk	0.64	11.0							
9	Direct	25	13							
	Salting-out	30.2	12.1						1.14	0.56
	SPE-Cart.	31.2	12.7						1.50	0.79
	SPE-Disk	27.5	11.0						1.34	0.79
10	Direct									
	Salting-out	0.33	7.12							
	SPE-Cart.	0.62	8.23							
	SPE-Disk	0.26	7.60							
14	Direct		13							
	Salting-out		5.98							
	SPE-Cart.		12.0							
	SPE-Disk		11.6							
16	Direct		40							
	Salting-out	0.58	28.7			0.04			0.39	0.13
	SPE-Cart.	0.77	33.8			0.03			0.43	0.17
	SPE-Disk	0.66	32.7			0.03			0.44	0.22
18	Direct	165	58						9	7
	Salting-out	141	39.1			0.80	0.96		8.5	5.62

TABLE 9
(continued)

Sample	Technique	Analyte Concentration (µg/L)								
		HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
	SPE-Cart.	152	44.4			0.93	0.88		9.5	7.01
	SPE-Disk	138	40.9			0.90	0.99		9.3	6.03
19	Direct	173	76				17		59	54
	Salting-out	172	69.5			2.6	23.1	1.20	65.2	56.4
	SPE-Cart.	142	75.6		0.11	2.5	20.9	1.08	57.7	50.5
	SPE-Disk	136	72.7		0.11	2.4	20.3	1.23	55.0	48.0
21	Direct	252	157	5			110		47	65
	Salting-out	227	132	6.62	0.30		102		42.6	56.5
	SPE-Cart.	238	146	6.90	0.33		104		48.0	63.5
	SPE-Disk	226	141	6.45	0.31		102		47.0	61.8
22	Direct	218	40							
	Salting-out	201	35.9						2.20	1.90
	SPE-Cart.	203	36.5						2.74	2.24
	SPE-Disk	199	35.8						2.78	2.08
24	Direct									
	Salting-out	2.15	7.54							
	SPE-Cart.	2.47	8.91							
	SPE-Disk	2.34	8.84							
25	Direct									
	Salting-out									
	SPE-Cart.		0.59							
	SPE-Disk		0.63							
27	Direct	112	608	8			180		10	8
	Salting-out	82.8	429	4.45	0.79		137		7.71	6.20
	SPE-Cart.	91.0	510	9.53	0.90		149		8.25	7.67
	SPE-Disk	77.3	445	7.37	0.79		128		8.16	6.33

TABLE 9
(continued)

Sample	Technique	Analyte Concentration (µg/L)								
		HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
28	Direct	325	102				14		51	40
	Salting-out	290	87.5	0.37	0.10		13.9		42.3	33.5
	SPE-Cart.	319	109	0.87	0.17		22.0		56.2	45.0
	SPE-Disk	249	85.7	0.65	0.13		17.2		43.0	34.5
29	Direct									
	Salting-out									
	SPE-Cart.		0.43							
	SPE-Disk		0.28							
31	Direct									
	Salting-out									
	SPE-Cart.		0.21							
	SPE-Disk		0.23							
32	Direct									
	Salting-out									
	SPE-Cart.									
	SPE-Disk	0.38								

An additional 11 samples (11, 12, 13, 15, 17, 20, 23, 26, 30, 31, and 33) were analyzed in which none of the analytes were detected by any of the techniques. Therefore, the non-detect results are not shown here. Similarly, for those samples that are shown here, the fields are left blank for the analytes that were not detected.

All data are taken from Reference 10.

TABLE 10

RELATIVE PERCENT DIFFERENCE BETWEEN DUPLICATE SAMPLE ANALYSES

		Relative Percent Difference (%)								
Sample	Technique	HMX	RDX	TNB	DNB	DNA	TNT	24D	4A	2A
4	Direct	0	24							
	Salting-out	0	15		6	100	8		18	11
	SPE-Cart.	1	12			0	45		8	5
	SPE-Disk	3	8			0	17		2	1
29	Direct									
	Salting-out									
	SPE-Cart.		26							
	SPE-Disk		7							
LCS	Direct	1	0	0			1	1		
	Salting-out	4	4	4			3	3		
	SPE-Cart.	6	1	7			6	6		
	SPE-Disk	5	7	7			13	6		

All data are taken from Reference 10.

TABLE 11

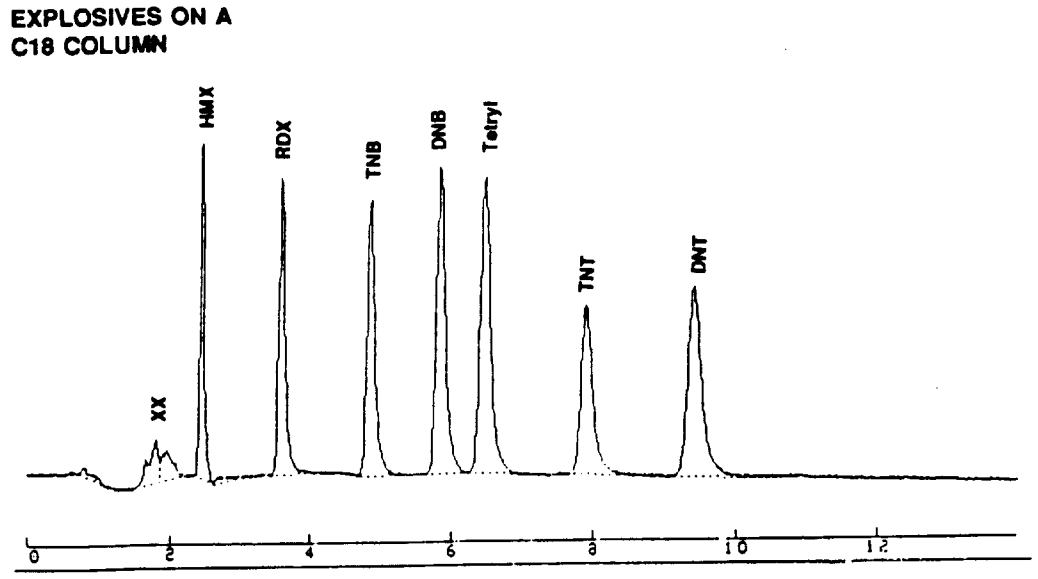
RECOVERY OF ANALYTES FROM SPIKED SAMPLES

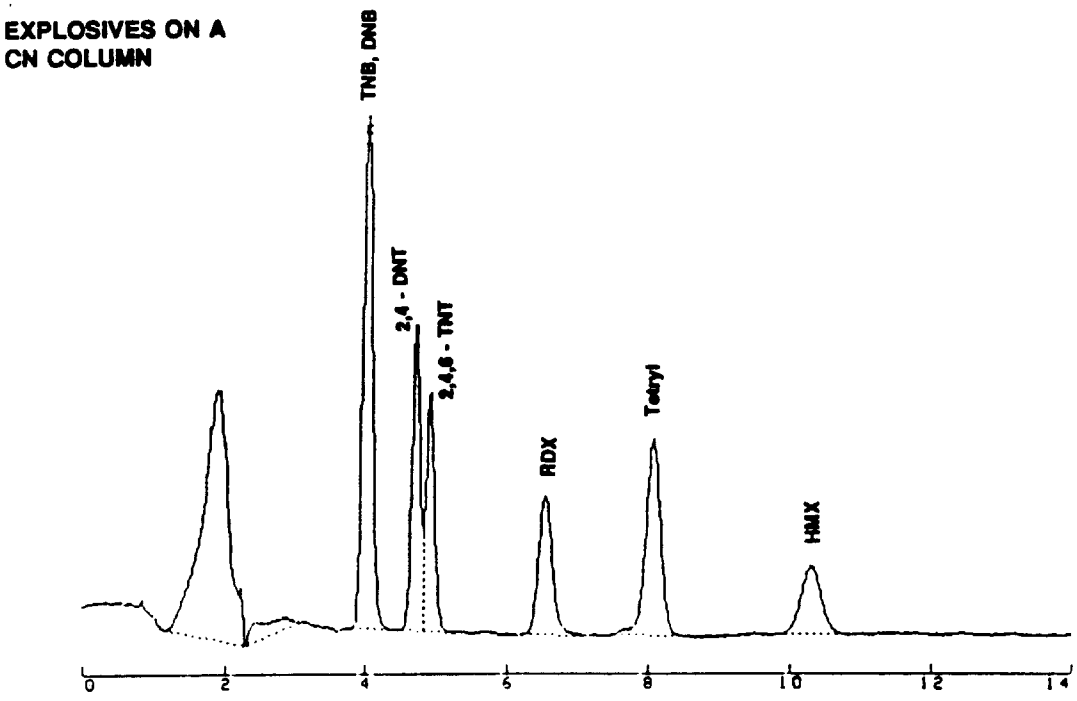
		Percent Recovery (%)				
Sample	Technique	HMX	RDX	TNB	TNT	24D
LCS1	Direct	99.5	98.5	95.6	96.5	98.1
	Salting-out	94.2	91.2	92.9	83.2	92.1
	SPE-Cart.	99.0	101.0	96.6	94.1	95.1
	SPE-Disk	92.5	95.6	89.3	88.6	86.9
LCS2	Direct	98.8	98.2	95.9	97.2	99.2
	Salting-out	91.0	95.0	89.0	81.0	89.0
	SPE-Cart.	93.5	100.0	83.0	89.1	89.3
	SPE-Disk	88.0	102.0	83.0	78.0	82.0
29	Direct	95.0	95.5	95.2	92.8	93.0
	Salting-out	107.0	89.0	85.0	89.0	65.0
	SPE-Cart.	103.0	107.0	104.0	05.0	102.0
	SPE-Disk	80.0	78.0	76.0	78.0	77.0
4	Direct	105.5	105.0	103.0	104.0	105.0
	Salting-out	23*	191*	76.0	83.0	76.0
	SPE-Cart.	351*	95*	92.2	91.1	93.7
	SPE-Disk	308*	49.5*	87.4	85.6	90.8

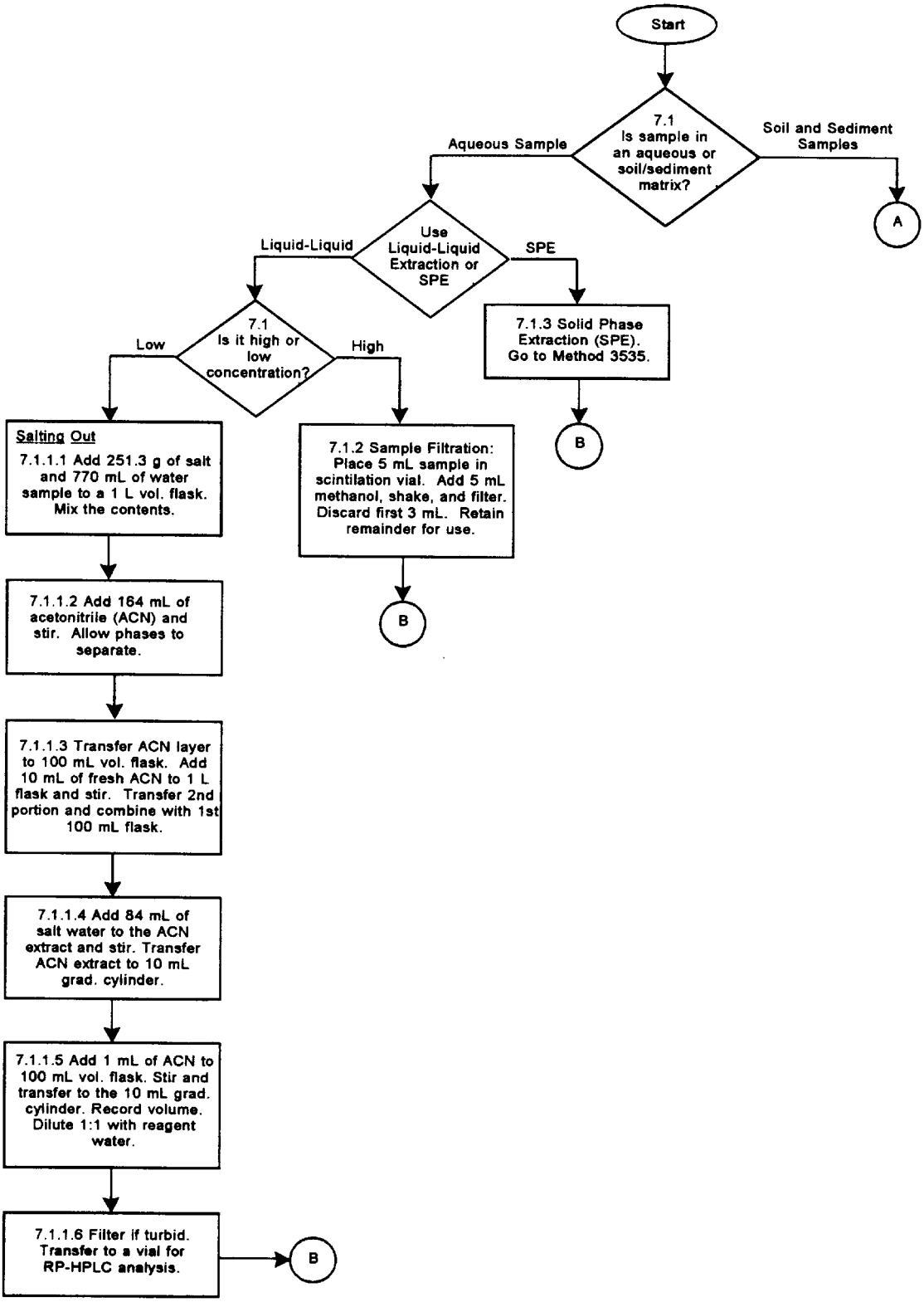
All data are taken from Reference 10.

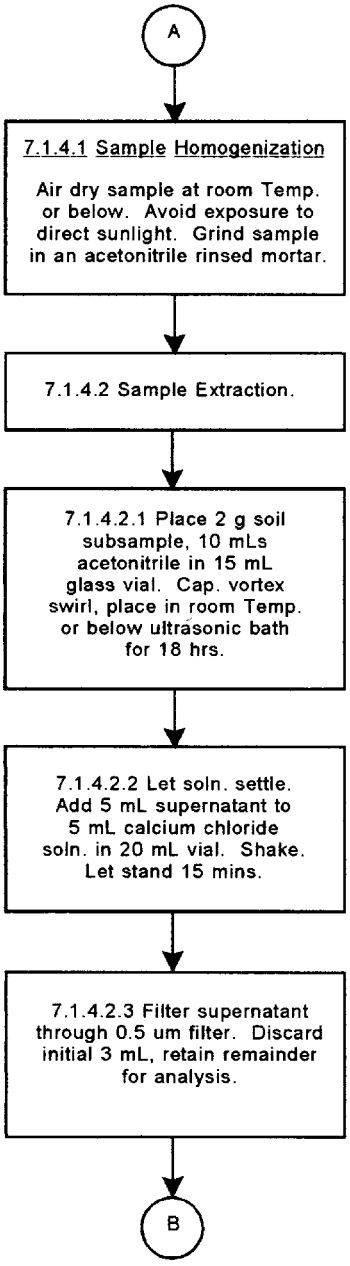
* Results for these analytes in Sample 4 are believed to result from spiking levels that are very similar to the background concentrations of these analytes in this sample (see Reference 10).

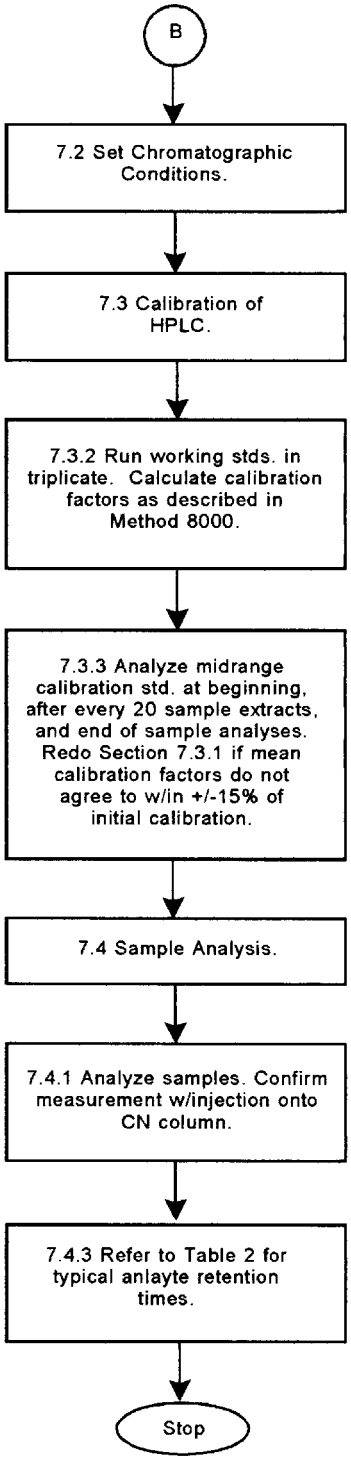
FIGURE 1
EXAMPLE CHROMATOGRAMS






Start
7.1 Is sample in an aqueous or soil/sediment matrix?
Aqueous Sample
Soil and Sediment Samples
A
Use Liquid-Liquid Extraction or SPE
Liquid-Liquid
SPE
7.1 Is it high or low concentration?
Low
High
7.1.3 Solid Phase Extraction (SPE). Go to Method 3535.
B
Salting Out
7.1.1.1 Add 251.3 g of salt and 770 mL of water sample to a 1 L vol. flask. Mix the contents.
7.1.2 Sample Filtration: Place 5 mL sample in scintilation vial. Add 5 mL methanol, shake, and filter. Discard first 3 mL. Retain remainder for use.
B
7.1.1.2 Add 164 mL of acetonitrile (ACN) and stir. Allow phases to separate.
7.1.1.3 Transfer ACN layer to 100 mL vol. flask. Add 10 mL of fresh ACN to 1 L flask and stir. Transfer 2nd portion and combine with 1st 100 mL flask.
7.1.1.4 Add 84 mL of salt water to the ACN extract and stir. Transfer ACN extract to 10 mL grad. cylinder.
7.1.1.5 Add 1 mL of ACN to 100 mL vol. flask. Stir and transfer to the 10 mL grad. cylinder. Record volume. Dilute 1:1 with reagent water.
7.1.1.6 Filter if turbid. Transfer to a vial for RP-HPLC analysis.
B


A
7.1.4.1 Sample Homogenization
Air dry sample at room Temp. or below. Avoid exposure to direct sunlight. Grind sample in an acetonitrile rinsed mortar.
7.1.4.2 Sample Extraction.
7.1.4.2.1 Place 2 g soil subsample, 10 mLs acetonitrile in 15 mL glass vial. Cap. vortex swirl, place in room Temp. or below ultrasonic bath for 18 hrs.
7.1.4.2.2 Let soln. settle. Add 5 mL supernatant to 5 mL calcium chloride soln. in 20 mL vial. Shake. Let stand 15 mins.
7.1.4.2.3 Filter supernatant through 0.5 um filter. Discard initial 3 mL, retain remainder for analysis.
B


B
7.2 Set Chromatographic Conditions.
7.3 Calibration of HPLC.
7.3.2 Run working stds. in triplicate. Calculate calibration factors as described in Method 8000.
7.3.3 Analyze midrange calibration std. at beginning, after every 20 sample extracts, and end of sample analyses. Redo Section 7.3.1 if mean calibration factors do not agree to w/in +/-15% of initial calibration.
7.4 Sample Analysis.
7.4.1 Analyze samples. Confirm measurement w/injection onto CN column.
7.4.3 Refer to Table 2 for typical anlayte retention times.
Stop